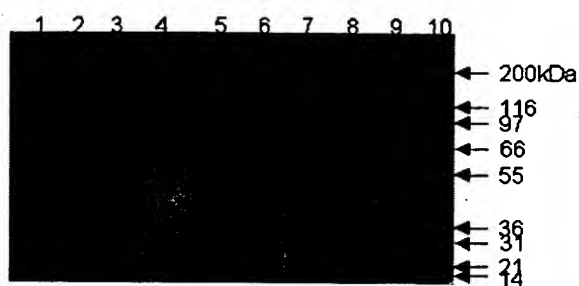
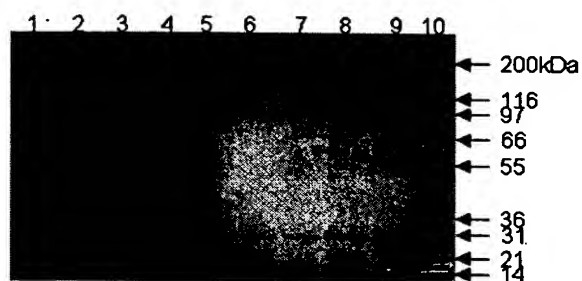


Fig. 1



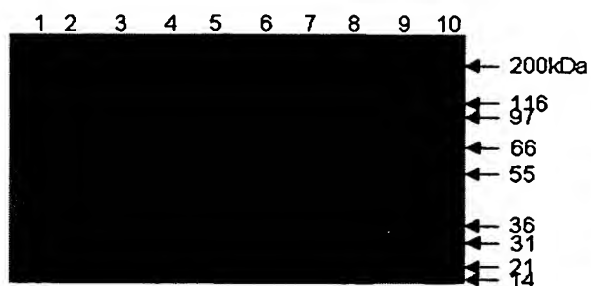
Lanes 1 & 10, marker proteins; lane 2 untreated mbh; lane 3, 50°C; lane 4, 60°C; lane 5, 70°C; lane 6, 80°C; lane 7, 90°C; lane 8, 100°C; lane 9, Protease M.

Fig. 2



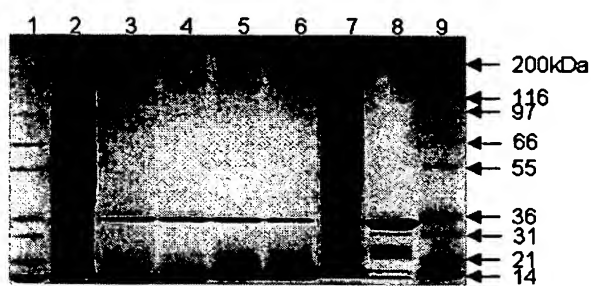
Lanes 1 & 10, marker proteins; lane 2 untreated mbh; lane 3, pH2; lane 4, pH4; lane 5, pH6; lane 6, pH8; lane 7, pH10; lane 8, pH12; lane 9, Protease M.

Fig. 3



Lanes 1 & 10, marker proteins;
lane 2 untreated mbh; lanes 3 - 8,
Rokko digest (20mg.ml^{-1} -
 0.1mg.ml^{-1}), lane 9, Rokko
(1mg.ml^{-1}).

Fig. 4



Lanes 1 & 9, marker proteins; lane 2
untreated mbh; lane 3, 2% SDS; lane
4, 1% SDS; lane 5, 0.5% SDS; lane 6,
0.25% SDS; lane 7, mbh + 2% SDS;
lane 8, Rokko (20mg.ml^{-1}).

Fig. 5

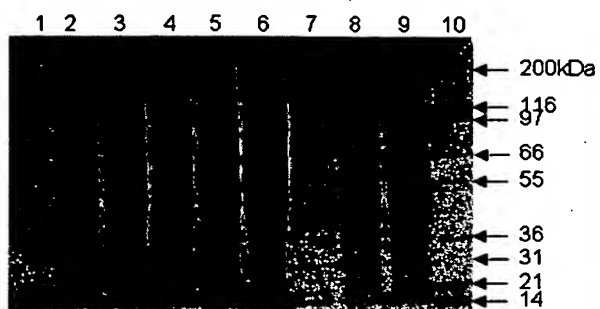
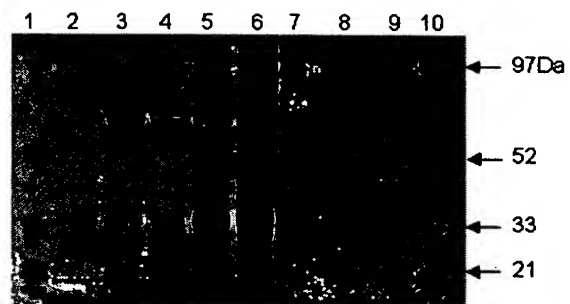


Fig. 6



Lanes 1 & 10, marker proteins; lanes 2 & 3, mbh; lanes 4 - 6, mbh pellet; lanes 7 - 9, mbh supernatant.

Fig.7

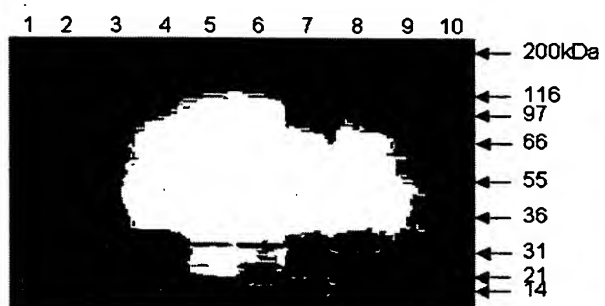


Fig.8



Lanes 1 & 10, marker proteins; lane 2, untreated mbh; lane 3, Protease G digest; lane 4, Protease G; lane 5, Protease R digest; lane 6, Protease R; lane 7, Protease C digest; lane 8, Protease C; lane 9, rec. mouse PrP.

1

2

3

4

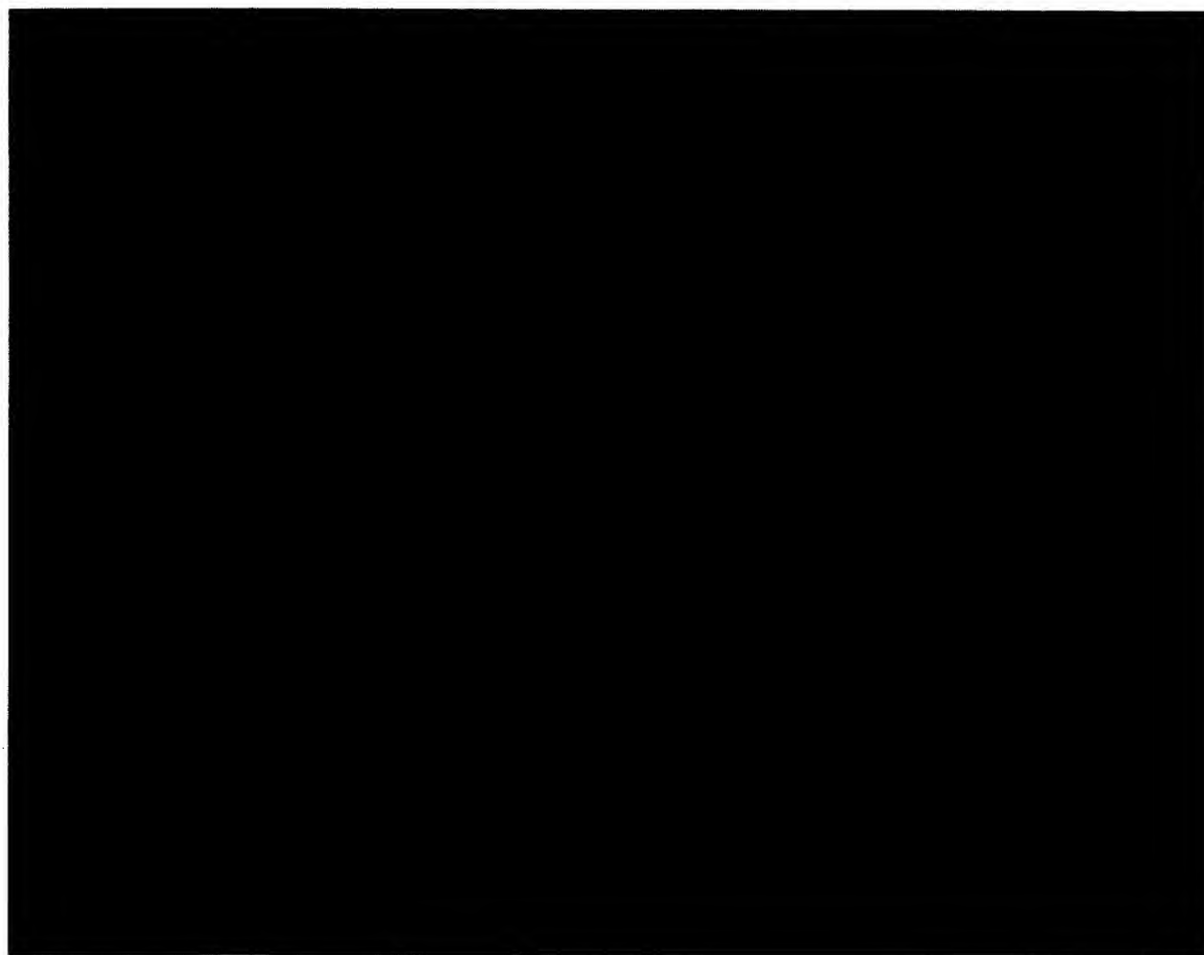


Fig. 9

5

6

7

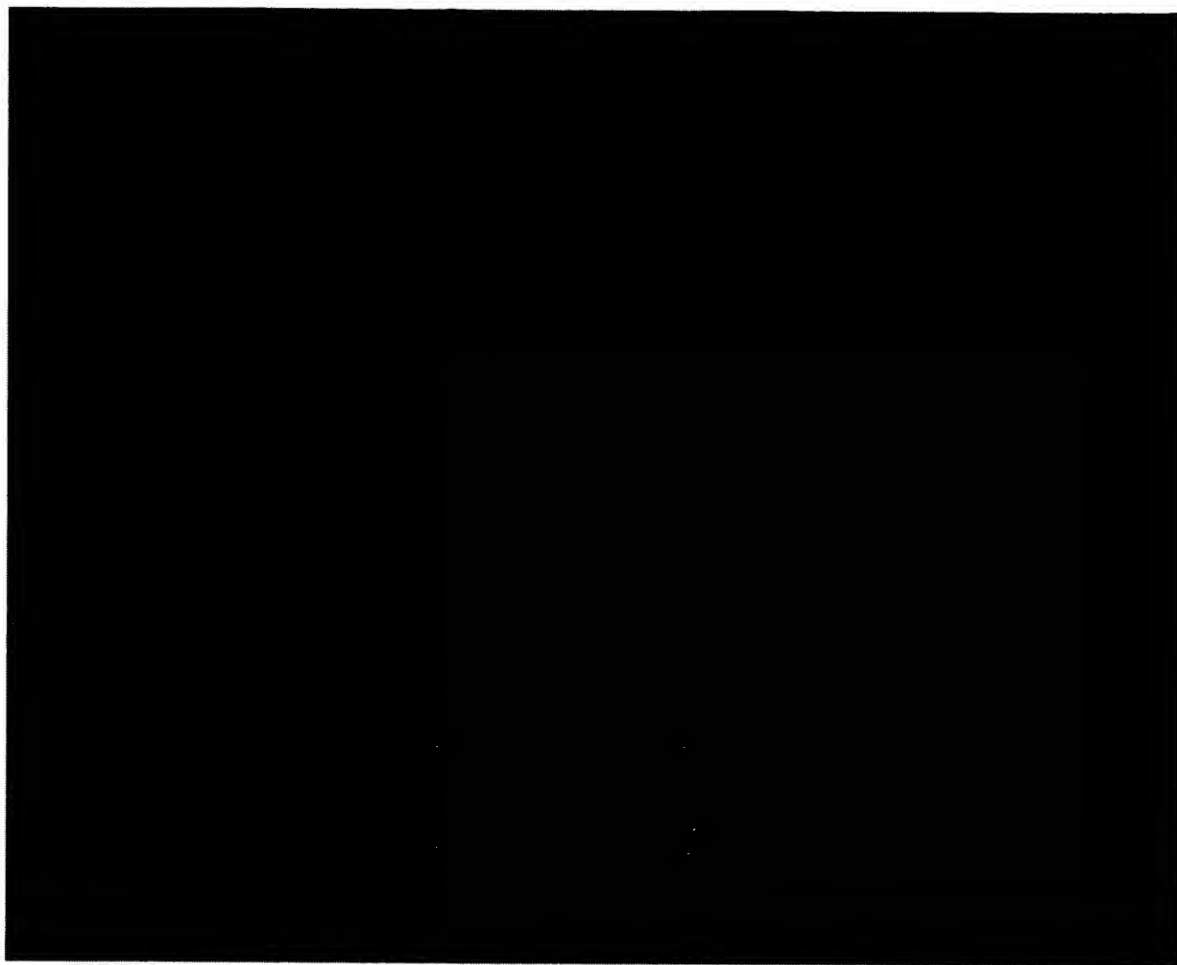


Fig. 10

1

2

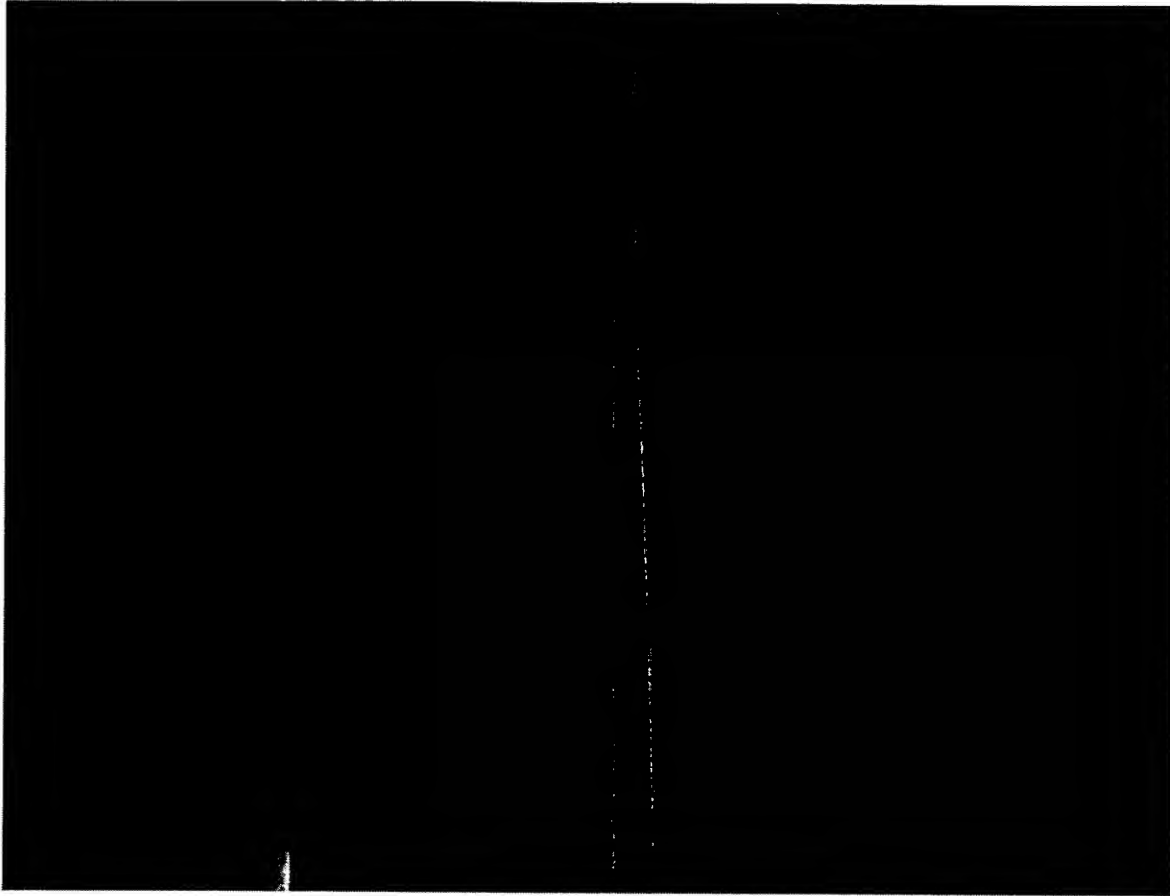


Fig. 11

3

7

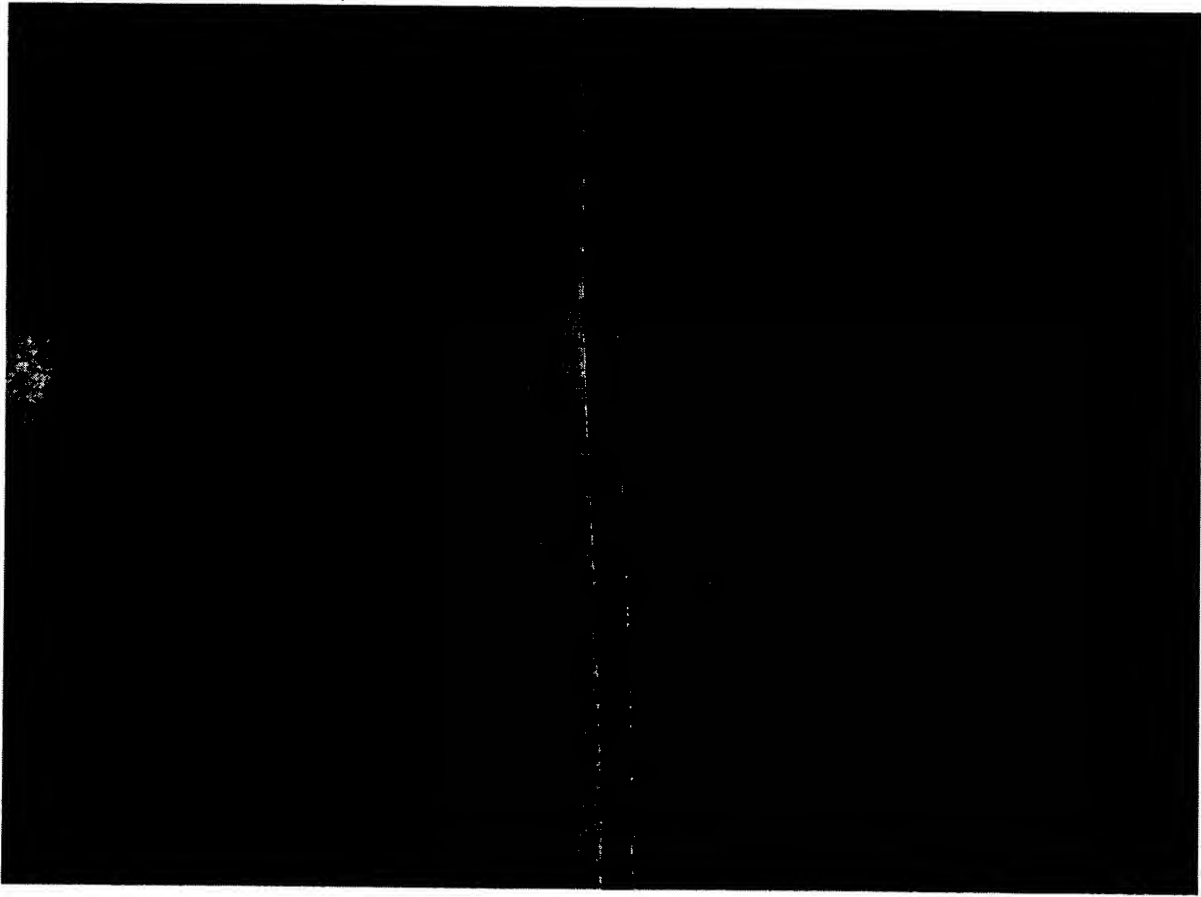


Fig. 12

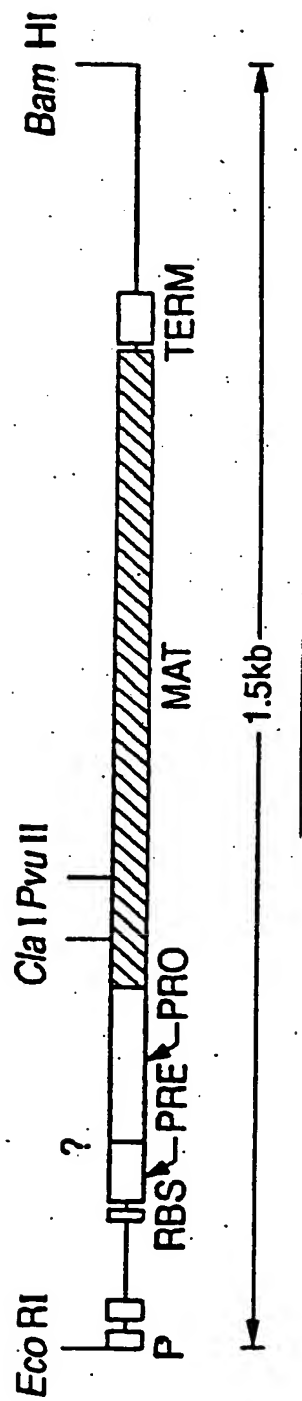


FIGURE 13.A

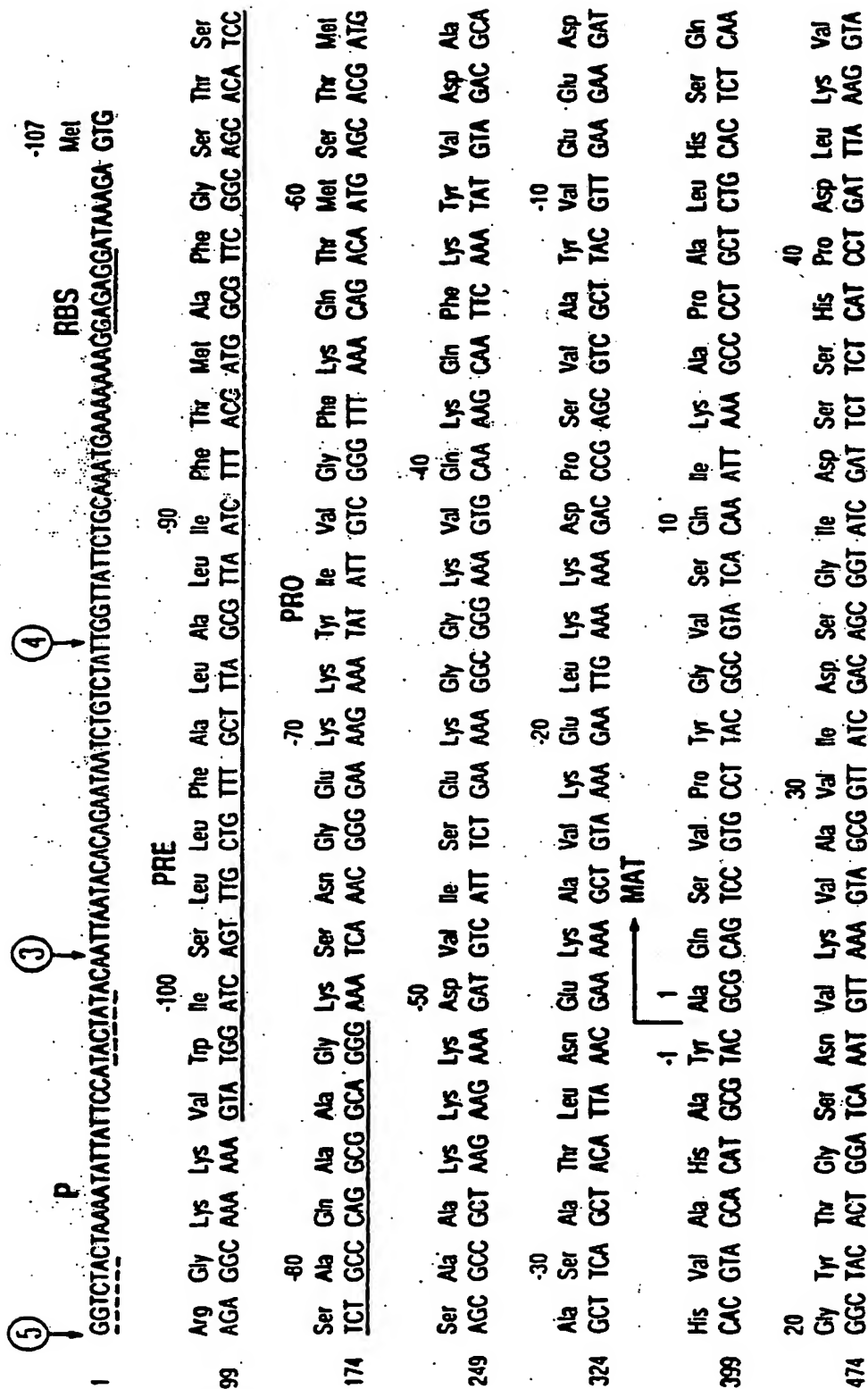


FIGURE 13.B1

50 Pro Asn 50 Asp
 Ala Gly Gly Ala Ser Met Val Pro Ser Glu Thr Asn Pro Phe Gln Asp Asp Asn Ser His Gly Thr His Val Ala
 549 GCA GGC GGA GCC AGC ATG GTT CCT TCT TCT GAA ACA AAT CCT TTC CAA GAC AAC AAC TCT CAC GGA ACT CAC GTT GGC

70 Gly Thr Val Ala Ala Leu Asn Asn Ser Ile Gly Val Leu Gly Val Ala Pro Ser Ala Ser Leu Tyr Ala Val Lys
 624 GGC ACA GTT GCG GCT CTT AAT AAC TCA ATC GGT GTA TTA GGC GTT GCG CCA AGC GCA TCA CTT TAC GCT GTA AAA

100 Asp Ala 110
 Val Leu Gly Ala Asp Gly Ser Gly Gln Tyr Ser Thr Ile Ile Asn Gly Ile Glu Trp Ala Ile Ala Asn Asn Met
 699 GTT CTC GGT GCT GAC GGT TCC GGC TCC GGC CAA TAG AGC TGG ATC ATT AAC GGA ATC GAG TGG GCG ATC GCA AAC AAT ATG

120 Asp Val Ile Asn Met Ser Leu Gly Gly Pro Ser Gly Ser Ala Ala Leu Lys Ala Val Asp Lys Ala Val Ala
 774 GAC GTT ATT AAC ATG AGC CTC GGC GGA CCT TCT GGT TCT GCT GCT TTA AAA GCG GCA GTT GAT AAA GCC GTT GCA

150 Ser Thr 160
 Ser Gly Val Val Val Val Ala Ala Ala Gly Asn Glu Gly Thr Ser Gly Ser Ser Thr Val Gly Tyr Pro Gly
 849 TCC GGC GTC GTA GTC GTT GCG GCA GCC GGT AAC GAA GGC ACT TCC GGC AGC TCA AGC ACA GTG GGC TAC CCT GGT

170 Lys Tyr Pro Ser Val Ile Ala Val Gly Ala Val Asp Ser Ser Asn Gln Arg Ala Ser Phe Ser Ser Val Gly Pro
 924 AAA TAC CCT TCT TCT ATT GCA GTA GGC GCT GTT GAC AGC AGC AAC CAA AGA GCA TCT TTC TCA AGC GTA GGA CCT

200 Glu Leu Asp Val Met Ala Pro Gly Val Ser Ile Gln Ser Thr Leu Pro Gly Asn Lys Tyr Gly Ala Tyr Asn Gly
 999 GAG CTT GAT GTC ATG GCA CCT GGC GTA TCT ATC CAA AGC ACG CTT CCT GGA AAC AAA TAC GGG GCG TAC AAC GGT

220 Thr Ser Met Ala Ser Pro His Val Val Ala Gly Ala Ala Leu Ile Leu Ser Lys His Pro Asn Trp Thr Asn Thr
 1074 ACG TCA ATG GCA TCT CCG CAC GTT GCC GGA GCG GCT TGT ATT CTT TCT TGT AAG CAC CCG AAC TGG ACA AAC ACT

Figure 13.82

1149	CAA	GTC	CGC	AGC	AGT	TTA	GAA	AAC	ACC	ACT	ACA	AAA	CTT	GGT	GAT	TCT	TTC	TAC	TAT	GGA	AAA	GGG	CTG	ATC	AAC
	Gln	Val	Arg	Ser	Ser	Leu	Glu	Asn	Thr	Thr	Thr	Lys	Leu	Gly	Asp	Ser	Phe	Tyr	Tyr	Gly	Lys	Gly	Leu	Ile	Asn
250	Gln																								
270																									
1224	GTA	CAG	CGC	GCA	GCT	CAG	TAA	<u>AACATATAAAACCGCCCTTGCGCCCGCGGTTTTTATTTTCTTCCTCCGCATGTTCAATCGCGTCC</u>																	
	Val	Gln	Ala	Ala	Ala	Gln	OC																		
								TERM																	
1316	ATA	TCC	GAC	GGAT	GGCT	CTCT	GAATA	TTTTTA	ACG	GAA	ACGG	GGG	TTG	ACCC	GGCT	CAGT	CCCG	TAC	GGCC	CAAG	TCC	TGA	ACG	CTC	CAATCGCGG
	Val	Gln	Ala	Ala	Ala	Gln	OC																		
1416	CTT	CCCG	GTTC	CGGT	CAG	CTCA	ATGCC	GTAC	CGGT	CGGG	GGTT	TCCT	GAT	ACCG	GGAG	ACGG	CATT	CGTA	TCGG	ATC					
	Val	Gln	Ala	Ala	Ala	Gln	OC																		

figure 13.83

CONSERVED RESIDUES IN SUBTILISINS FROM
BACILLUS AMYLOLIQUEFACIENS

```

1      10      20
A Q S V P . G . . . . . A P A . H . . G

21     30     40
. T G S . V K V A V . D . G . . . . H P

41     50     60
D L . . . G G A S . V P . . . . . Q D

61     70     80
. N . H G T H V A G T . A A L N N S I G

81     90     100
V L G V A P S A . L Y A V K V L G A . G

101    110    120
S G . . S . L . . G . E W A . N . . . .

121    130    140
V . N . S L G . P S . S . . . . . A . .

141    150    160
. . . . . G V . V V A A . G N . G . . .

161    170    180
. . . . . Y P . . Y . . . . . A V G A .

181    190    200
D . . N . . A S P S . . G . . L D . . A

201    210    220
P G V . . Q S T . P G . . Y . . . . N G T

221    230    240
S M A . P H V A G A A A L . . . . K . . .

241    250    260
W . . . Q . R . . L . N T . . . . L G . .

261    270
. . Y G . G L . N . . A A . .

```

FIGURE 14

COMPARISON OF SUBTILISIN SEQUENCES FROM:

B. amyloliquefaciens

B. subtilis

B. licheniformis

B. lentus

01	10	20	30
1 Q S V P Y G V S Q I K A P A L H S Q G Y T G S S N V K V A V I D S G I D S S H P			
A Q S V P Y G I S Q I K A P A L H S Q G Y T G S S N V K V A V I D S G I D S S H P			
A Q T V P Y G I P L I K A D K V Q A Q G F K G A N V K V A V L D T G I Q A S H P			
A Q S V P W G I S R V Q A P A A H N R G L T G S G V K V A V L D T G I S T * H P			
41	50	60	70
D L K V A G G A S M V P S E T N P F Q D N N S H G T H V A G T V A A L N N S I G			
D L N V R G G A S F V P S E T N P Y Q D G S S H G T H V A G T I A A L N N S I G			
D L N V V G G A S F V A G E A Y N * T D G N G H G T H V A G T V A A L D N T T G			
D L N I R G G A S F V P G E * P S T Q D G N G H G T H V A G T I A A L N N S I G			
81	90	100	110
V L G V A P S A S L Y A V K V L G A D G S G Q Y S W I I N G I E W A I A N N M D			
V L G V S P S A S L Y A V K V L D S T G S G Q Y S W I I N G I E W A I S N N M D			
L G V A P S V S L Y A V K V L N S S G S G S Y S G I V S G I E W A T T N G M D			
V L G V A P S A E L Y A V K V L G A S G S G S V S S I A Q G L E W A G N N G M H			
121	130	140	150
V I N M S L G G P S G S A A L K A A V D K A V A S G V V V V A A A A G N E G T S G			
V I N M S L G G P T G S T A L K T V V D K A V S S G I V V V A A A A G N E G S S G			
V I N M S L G G A S G S T A M K Q A V D N A Y A R G V V V V A A A A G N S G N S G			
V A N L S L G S P S P S A T L E Q A V N S A T S R G V L V V A A S G N S G A G S			

Figure 15.A

161. 170 180 190
 S S S T V G Y P G K Y P S V I A V G A V D S S N Q R A S F S S V G P E L D V M A
 S T S T V G Y P A K Y P S T I A V G A V N S S N Q R A S F S S A G S E L D V M A
 S T N T I G Y P A K Y D S V I A V G A V D S S N S N R R A S F S S V G A E L E V M A
 * * I S Y P A R Y A N A M A V G A T D Q N N R R A S F S Q Y G A G L D I V A

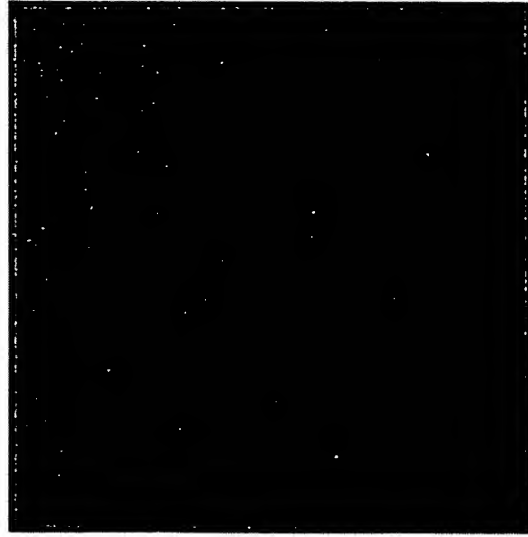
201 210 220 230
 P G V S I Q S T L P G N K Y G A Y N G T S M A S P H V A G A A A L I L S K H P N
 P G V S I Q S T L P G G T Y G A Y N G T S M A T P H V A G A A A L I L S K H P T
 P G A G V Y S T Y P P T N T Y A T L N G T S M A S P H V A G A A A L I L S K H P N
 P G V N V Q S T Y P G S T Y A S L N G T S M A T P H V A G A A A L V K Q K N P S

241 250 260 270
 W T N T Q V R S S L E N T T T K L G D S F Y Y G K G L I N V Q A A A Q
 W T N A Q V R D R L E S T A T Y L G N S F Y Y G K G L I N V Q A A A Q
 L S A S Q V R N R L S S T A T Y L G S S F Y Y G K G L I N V E A A A Q
 W S N V Q I R N N H L K N T A T S L G S T N L Y G S G L V N A E A A T R

Figure 15.B

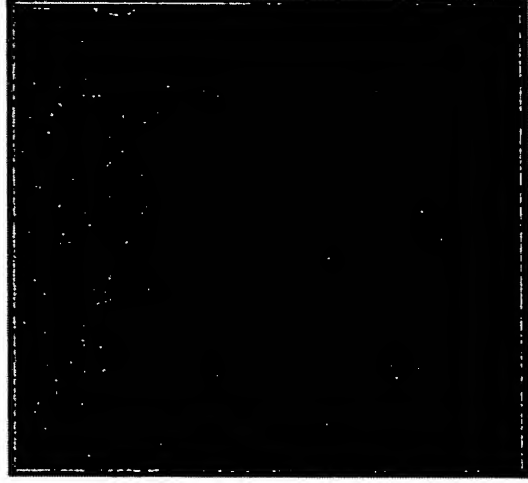
Initial evaluation results

MC-A



m mbh 2 4 6 8 10 12 P m

MC-3



m mbh 2 4 6 8 10 12 P m

MC-4

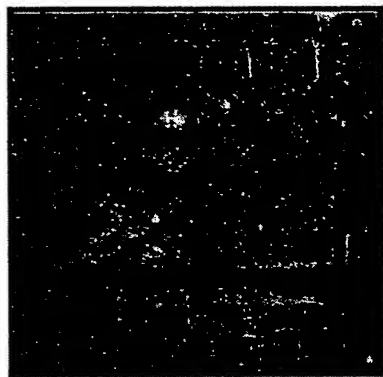


m mbh 2 4 6 8 10 12 P m

Fig. 16

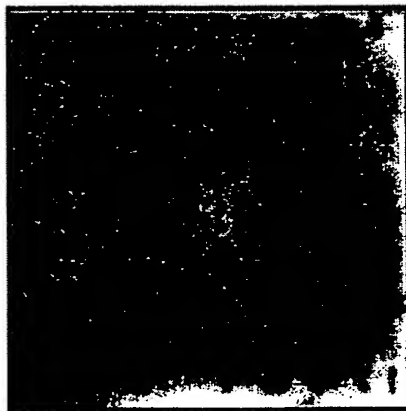
Comparison with Properase

Properase 60°C 30 minutes



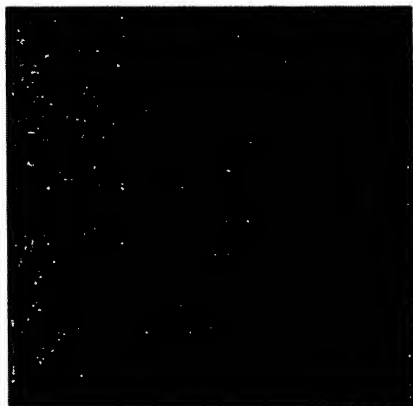
m 2 4 6 8 10 12 P rPrP m

MC-A 50°C 30 minutes



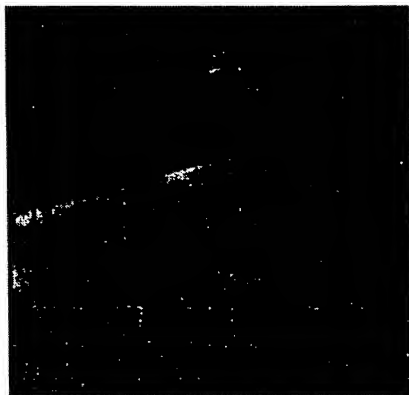
m 2 4 6 8 10 12 P rPrP m

MC-3 50°C 30 minutes



m 2 4 6 8 10 12 P rPrP m

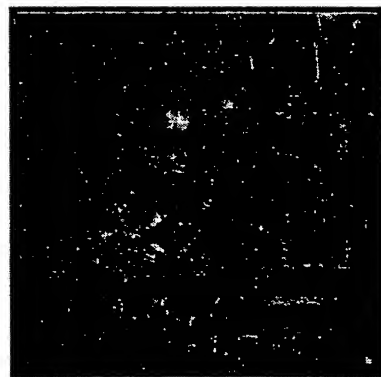
MC-4 50°C 30 minutes



m mbh 2 4 6 8 10 12 P m

Fig. 17

Comparison with Properase



Properase 60°C 30 minutes

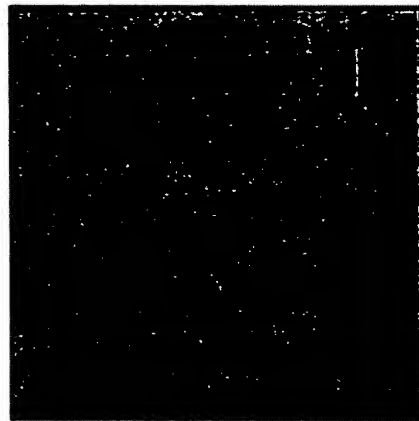
m 2 4 6 8 10 12 P rPrP m

MC-A 60°C 30 minutes



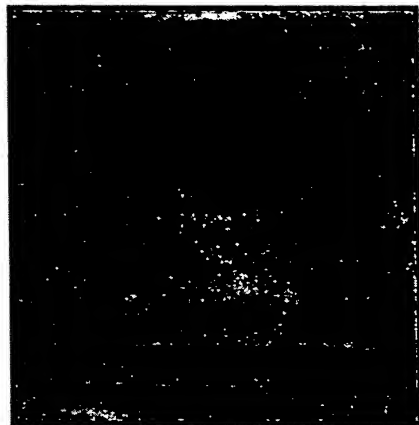
m 2 4 6 8 10 12 P rPrP m

MC-3 60°C 30 minutes



m 2 4 6 8 10 12 P rPrP m

MC-4 60°C 30 minutes

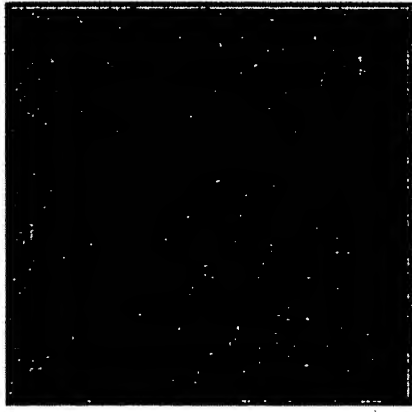


m 2 4 6 8 10 12 P rPrP m

Fig. 18

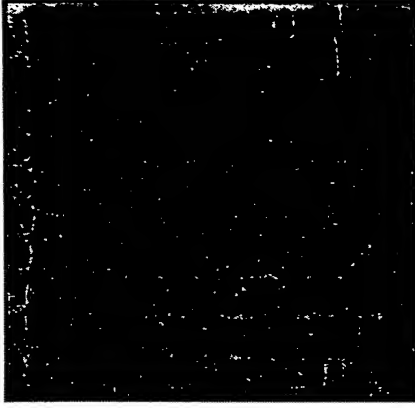
Temperature profiling with MC-3

50°C 30 minutes



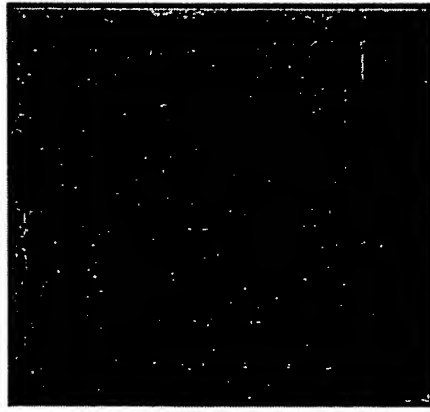
m 2 4 6 8 10 12 P rPrP m

70°C 30 minutes



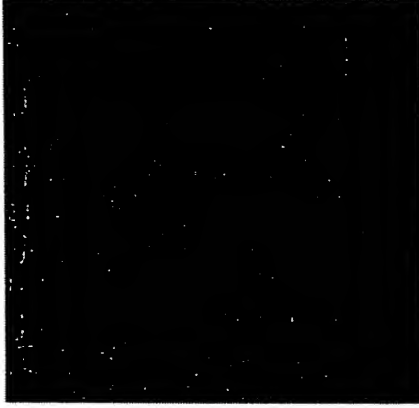
m 2 4 6 8 10 12 P rPrP m

60°C 30 minutes



m 2 4 6 8 10 12 P rPrP m

80°C 30 minutes



m 2 4 6 8 10 12 P rPrP m

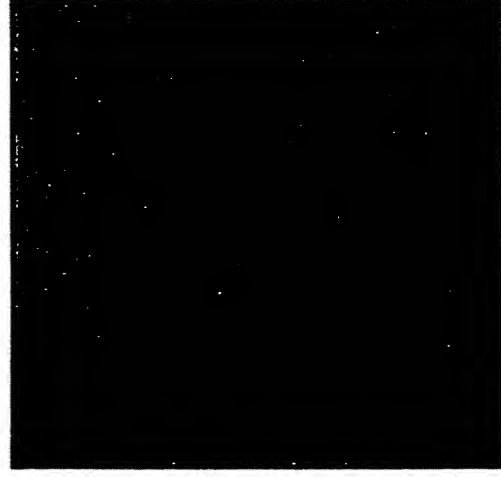
Fig. 19

Detection with PAb2

mbh pH 2-12 digested at 50°C 30 minutes

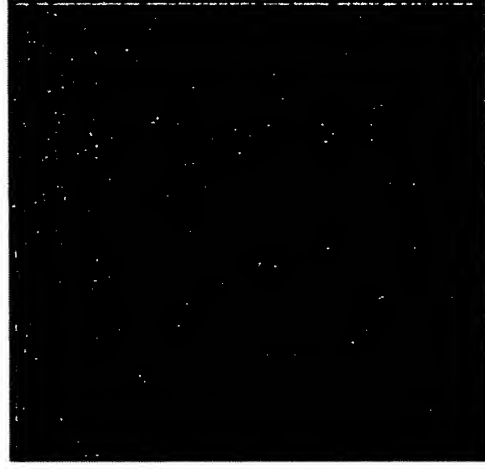
- Detected with a chemiluminescent detection substrate (Pierce)

MC-A



m 2 4 6 8 10 12 P rPrP m

MC-3



m 2 4 6 8 10 12 P rPrP

MC-4



m mbh 2 4 6 8 10 12 P m

Fig. 20

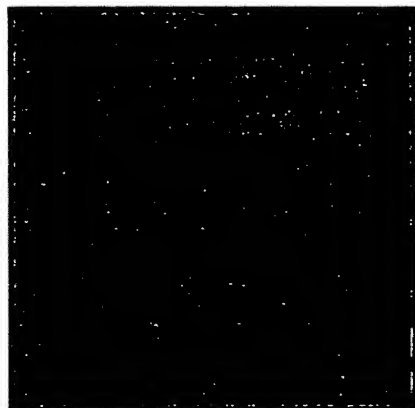
MC-3 dilutions at pH 10 & pH 12

6H4 West Dura



m
rPrP
P
1:100
1:50
1:20
1:4
n
mbh
m

PAb2 West Dura



m
rPrP
P
1:100
1:50
1:20
1:4
n
mbh
m

pH 10

Monomer bands at
1:20 dilution

HMW bands across
dilution range

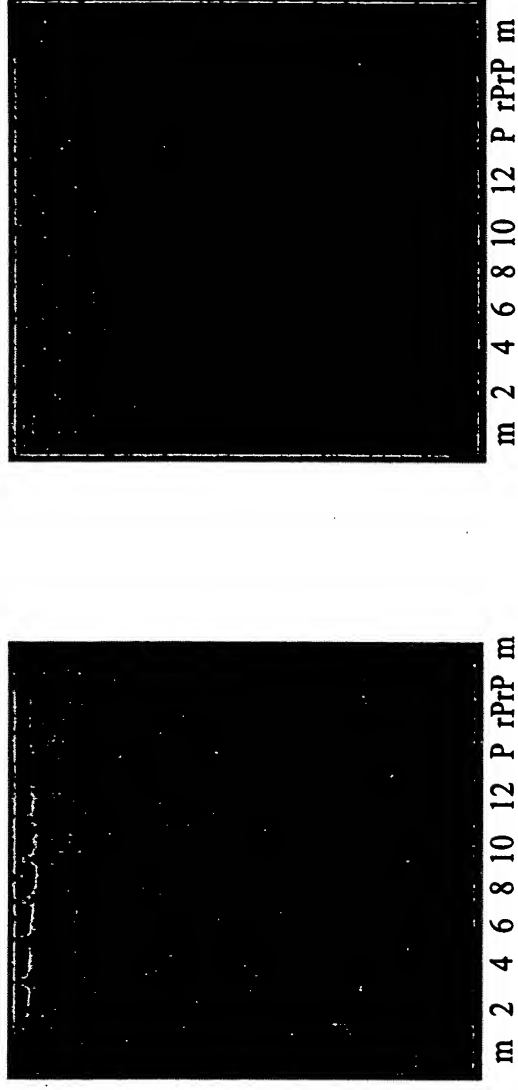
pH 12

No monomer bands

HMW bands much
reduced across
dilution range

Fig. 21

Comparison with Proteinase K



- Characteristic PrP^{Sc} monomer bands pH 2-10
Incomplete digestion pH 12 however no clear monomers
HMW bands present pH 2-12
The new proteases are better at removing both the
monomer and HMW bands than Proteinase K

Fig. 22